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Fine-Structure Deletion Map and Complementation Analysis of the *glnA-glnL-glnG* region in *Escherichia coli*

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A total of 399 independent mutants of *Escherichia coli* were obtained which have point and insertion mutations in the *glnA* region. Mutants isolated included Gln^- and Reg^- strains (unable to utilize arginine as a nitrogen source). Mutations were mapped with 73 deletion-containing derivatives of a λ *gln* phage. Complementation analysis was performed with λ *gln* derivatives containing point mutations which conferred a Gln^- or Reg^- phenotype. Deletion mapping and complementation analysis assigned 104 mutations in 24 deletion intervals to *glnA*. Mutations in Reg^- strains were assigned to two genes, *glnL* and *glnG*. *glnL* contained 131 mutations in 12 deletion intervals, and *glnG* contained 164 mutations in 10 deletion intervals. The gene order is *glnA-glnL-glnG*, transcribed from left to right. Polarity of insertion mutations indicates that *glnL* and *glnG* form an operon. Complementation analysis of *glnA* insertion mutations with *glnL* and *glnG* mutations showed polarity of *glnA* onto most *glnL* and *glnG* alleles, suggesting that transcription of *glnA* may proceed into the *glnL-glnG* operon. All mutations analyzed in *glnA* conferred a Gln^- phenotype. However, we also found that over half of the Gln^- strains isolated after chemical mutagenesis contained point mutations in *glnG*. Mutants which synthesized a high level of glutamine synthetase in the presence of ammonia (GlnC phenotype) were selected as revertants of a strain with a Tn*10* insertion in *glnD* and were mapped with chromosomal deletions. Results indicate that mutations in 12 of 15 examined strains clearly map outside of *glnA*, probably in *glnL*.

In enteric bacteria, the *glnA* gene codes for glutamine synthetase (GS), the enzyme responsible for the biosynthesis of glutamine and, together with glutamate synthase, for the assimilation of ammonia into glutamate (11). The level of synthesis of glutamine synthetase and many other proteins necessary for utilization of various nitrogenous compounds is increased in response to nitrogen limitation. Many reports have concluded that glutamine synthetase is responsible for regulating its own synthesis and the synthesis of other proteins involved in nitrogen metabolism (2, 3, 10, 27, 28, 31). This conclusion is based largely on the existence of mutants having a variety of phenotypes with mutations linked to and presumed to be in *glnA*. These mutants included glutamine auxotrophs (Gln^-) (2, 3, 27), constitutive mutants which synthesized high levels of GS and histidase in the presence of ammonia (GlnC) (2, 3, 9, 27), and mutants which were Gln^+ but produced low levels of GS under all conditions and failed to derepress histidase (GlnR or Reg^-) (10).

The regulatory role of the GS protein has been questioned, since mutations conferring the Reg^- (GlnR) phenotype have now been found in a separate gene, described as *glnG* in *Escherichia coli* (20) and *glnR* in *Salmonella typhimurium* (15). Recently, this gene has been redefined as two cistrons designated *ntrB* and *ntrC* (17). Those strains studied with mutations in these two genes failed to grow on the poor nitrogen source arginine and suppressed the Gln^- phenotype of *glnF* strains (17).

The study of GS and nitrogen regulation is seriously hampered by the lack of a thorough genetic characterization of the *glnA* region. In this study, we performed a detailed genetic analysis of Gln^- mutants, mutants unable to grow on poor nitrogen sources (Reg^-), and GlnC mutants.

A collection of deletion-containing derivatives was isolated among heat- and chelator-resistant mutants of a λ *gln*-specialized transducing phage. These phage were used to obtain a fine structure map of approximately 400 mutations. Complementation analysis was performed with point and insertion mutations, and the mutations from Gln^- and Reg^- strains were assigned to one of three genes, *glnA*, *glnL*, or *glnG*. The complementation pattern of polar mutations was used to determine the transcriptional organization of these three genes.

(Part of this work has been presented previ-

ously [T. MacNeil, D. MacNeil, and B. Tyler, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, K83, p. 151].)

MATERIALS AND METHODS

Chemicals and media. The rich medium was Luria broth containing 0.2% glutamine (LBg) (23). The nitrogen-free minimal medium was W salts (26). Sugars were added to a final concentration of 0.4%, and filter-sterilized nitrogen sources were added to 0.2%. GN minimal medium contained glucose and ammonium sulfate. GNg medium was GN with added glutamine. GA minimal medium contained glucose and arginine, and RNg medium contained rhamnose, ammonium sulfate, and glutamine. Tetracycline (used at 20 μg/ml), kanamycin (used at 25 μg/ml), and ampicillin (used at 50 μg/ml) were obtained from Sigma Chemical Co., St. Louis, Mo. Diethyl sulfate (DES) was obtained from Eastman Kodak Co., Rochester, N.Y.

Strains. All strains used in this study were derivatives of ET8000, except CBK049, CGSC5530, FS321, JC10240, ET6080, and ET6082, and are listed in Table 1. In addition to mutations isolated in ET8000 described here, mutations from 18 strains previously isolated (*glnA2*, *glnA200*, *glnA201*, *glnA202*, and *gln-1854* through *gln-1870*) were mapped. These mutations were transduced into ET8000 by P1, utilizing the close linkage of *zig-2*::Tn*10* to *glnA* (19). *glnA201* and *glnA202* are ethyl methane sulfonate induced (30). *gln-1854* through *gln-1860* are spontaneous mutations conferring a Gln$^-$ phenotype. *gln-1862* through *gln-1870* are hydroxylamine-induced mutations. Mutations *gln-1842*::Mu and *gln-1844*::Mu in the Reg$^-$ strains ET6080 and ET6082 (20), respectively, were also mapped.

Phage. Lysates of Mu d1 and P1 *vir* were prepared as described (7, 12). Lysates of λ *gln* and its deletion-containing derivatives were prepared by lytic growth on ET8000 and ET8051, respectively. NM811 was a gift of W. S. Kelley, Biogen, Inc., Cambridge, Mass. This phage contains the *glnA*, *L*, and *G* genes and was constructed by cloning *Hin*dIII-digested *E. coli* DNA into a *Hin*dIII λ cloning vector NM742 (*sr*Iλ1-2) *trpE* Δ(*att-red*) *imm*^{21} *nin5* (14). NM811 will be referred to as λ *gln*. λ *int*-h3 was a gift of D. Friedman, University of Michigan, Ann Arbor (18).

Isolation of mutants. (i) Gln$^-$ and Reg$^-$ mutants isolated by penicillin enrichment. Cultures of ET8000 were infected with Mu d1 (7) or mutagenized by DES (24). These cultures were subjected to a penicillin enrichment for strains unable to grow without glutamine (Gln$^-$) or unable to utilize arginine and proline as nitrogen sources (Reg$^-$), as previously described (20), with several modifications. After penicillin treatment, strains containing mutations *gln-1000* through *gln-1205* and *gln-1263* through *gln-1472* were isolated after outgrowth in glucose-glutamine medium, and strains containing mutations *gln-1520* through *gln-1672* were isolated after outgrowth in GNg. Both Gln$^-$ and Reg$^-$ strains were isolated from each mutagenized culture by plating the outgrowth on two different media. Gln$^-$ strains were identified as small colonies on 0.4% glucose–0.2% ammonium–0.002% glutamine medium, and Reg$^-$ mutants were identified as small colonies on 0.4% glucose–0.1% arginine–0.1% proline–0.005% aspartate medium. Gln$^-$ and Reg$^-$ strains with mutations in the *glnA* region were identified as mutants corrected by F′ 133 (*glnA*$^+$) and as strains which contained a mutation conferring the Gln$^-$ or Reg$^-$ phenotype linked to *zig*-2::Tn*10*, a Tn*10* insertion that is 80% P1 cotransducible with *glnA* (19). Mutations

TABLE 1. Strains

Strain	Genotype	Source
CBK049	*argG*::Tn*5*	C. Berg
CGSC5530	*glnA2 trpA9825 rpsL196*	B. Bachman
ET3617	*glnA202 lac gal hsdR*	30
ET6080	*gln-1842*::Mu *rhaD* Δ*lac-169 thi rpsL*[a]	20
ET6082	*gln-1844*::Mu *rhaD* Δ*lac-169 thi rpsL*[b]	20
ET8000	*rbs lacZ*::IS*1 gyrA hutC*$_K$[c]	S. Guterman
ET8040	*metB136*::Tn*5*	20
ET8045	*glnF208*::Tn*10*	20
ET8050	*gltB31*	21
ET8051	Δ(*glnA-rha*)VIII	20
ET8052	*glnF100*	20
ET8053	*glnD99*::Tn*10*	4
ET8056	*zig-2*::Tn*10*	19
ET8269	*glnG1206*::Tn*5*	25
ET8324	*glnA1262*::Tn*5*	S. Brom
ET10300	*metB136*::Tn*5 glnD99*::Tn*10* Δ(*rha-glnA*)*1693*	This laboratory
ET10573	*metB136*::Tn*5 glnD99*::Tn*10* Δ(*rha-glnG*)*1705*	This laboratory
ET10574	*metB136*::Tn*5 glnD99*::Tn*10* Δ(*rha-glnL*)*1711*	This laboratory
ET10575	*metB136*::Tn*5 glnD99*::Tn*10* Δ(*rha-glnL*)*1821*	This laboratory
FS321	*glnA200 his-1 argG metB*	27
JC10240	HfrPO45 *srlC300*::Tn*10 recA56 thr-300 ilv-318 rpsE300*	8

[a] Formerly *gln2*::Mu.

[b] Formerly *gln4*::Mu.

[c] Subscript K indicates that this gene is from *K. aerogenes*; it allows *E. coli* to grow on histidine as a nitrogen source, and the *hut* enzymes are expressed in the absence of inducer.

gln-1041, *-1042*, *-1051*, *-1054*, *-1077*, *-1078*, *-1080*, *-1082*, *-1083*, *-1098*, *-1131*, *-1142*, *-1167*, and *-1357* were isolated after penicillin enrichment of Mu d1-infected cultures but were subsequently shown not to contain a prophage at the *glnA* locus and are designated spontaneous. All Gln^- and Reg^- mutants were independent, since all mutants saved from a given selection had different phenotypes.

(ii) Suppressors of *glnF*. Suppressors of *glnF* mutations were generated by two methods. (i) Spontaneous Reg^- mutants were isolated by selection of Gln^+ revertants from a strain containing *glnF100* (20). P1 *vir* was used to transduce the *glnA*-linked suppressors into ET8051 Δ(*rha-glnA*)VIII (20) by selection for Gln^+ transductants. Cotransduction of the suppressor was identified by the inability of transductants to utilize arginine. All revertants contained *glnF* suppressor mutations which were P1 cotransducible with *glnA* at a frequency of greater than 90%. These mutations included *gln-1473* through *gln-1519*. (ii) Mu d1-induced *glnF* suppressors were isolated by Mu d1 infection of a strain containing *glnF*::Tn*10* and a closely linked mutation, *argG*::Tn*5*, followed by selection of Gln^+ revertants. To remove the *glnF* mutation, revertants were transduced with P1 *vir* grown on ET8000 to Arg^+ and scored for Tet^s, indicating loss of *glnF*::Tn*10*. Mu d1-induced *glnF* suppressors included mutations *gln-1783* through *gln-1806*.

(iii) GlnC mutants. GlnC mutants were isolated among spontaneous revertants of ET8050 (*gltB31*) and ET8053 (*glnD99*::Tn*10*). They were selected by plating 10^8 to 10^9 cells on GA medium. Revertants which produced GS constitutively at high levels in the presence of ammonium were identified by a colony test employing the γ-glutamyl transferase assay, in which colonies that produce GS at a depressed level turn a dark rust color and repressed colonies remain yellow (21). In the GlnC mutants studied here, the mutations responsible for the GlnC phenotype were tightly linked to *glnA*, since transduction of ET8051 Δ(*glnA-rha*)VIII to Gln^+ with P1 grown on GlnC strains yielded predominantly GlnC transductants. Thus, our mutants of the GlnC phenotype are defined as constitutive for *glnA* expression but have not been characterized for expression of histidase and other nitrogen-regulated genes.

Isolation of λ *gln* deletion phages. A modification of the method of Parkinson and Huskey (22) was used to isolate derivatives of λ *gln*-containing deletions. λ *gln* lysates were diluted 1:50 into 0.02 M Tris-hydrochloride (pH 7.5)–0.1 M NaCl with or without 0.01 M citrate. Incubation of the phage at 55°C for 30 min reduced viability by 10^{-4}. To increase the frequency of deletion mutants in the phage population, 1 ml of a treated lysate was regrown to high titer on ET8000, and this lysate was retreated at 55°C as described. The cycle was repeated for a series of three treatments at 55°C in the absence of Mg^{2+}. A total of 24 independent λ *gln* lysates were cycled by this method, 12 in the presence of 0.01 M citrate and 12 in its absence. However, the presence or absence of citrate did not affect the frequency or size of deletion mutants obtained. Surviving phage were plated on ET3617 on Luria broth plates without glutamine. Gln^+ phage formed turbid plaques on this Gln^- host owing to growth of Gln^+ lysogens in the glutamine-limited lawn, whereas Gln^- phage, such as NM742 (λ *trpE*) (14), formed clear plaques. Between 10 and 90% of surviving phage from the 24 cycled λ *gln* lysates formed clear plaques on ET3617. Three or four clear plaques were purified from each of the 24 lysates. All phage were tested for their ability to transduce several *gln* mutations, and only those which could be verified as independent were saved for further analysis. From lysates which had a high percentage of clear plaques several turbid plaques were examined to identify phages which might carry deletions not extending into *glnA*. λ *gln*99 was isolated from a turbid plaque and is Gln^+.

Mapping mutations in Gln^- and Reg^- strains. All *gln* mutations were mapped by crossing deletion-containing phages with strains carrying *gln* point and insertion mutations. LBg plates were spread with 0.1 ml of an overnight culture of each mutant. A 0.05-ml sample of five different λ deletion mutants (5×10^8 PFU/ml) was spotted on the lawn of recipient cells. After overnight incubation at 34°C, plates were replicated to selective media, GN for Gln^- strains and GA for Reg^- strains. Gln^+ and Reg^+ transductants were scored after incubation of selective plates for 2 and 4 days, respectively.

Isolation of λ *gln* phages carrying point mutations in *glnA*, *glnL*, or *glnG*. λ *gln* was diluted to obtain single plaques on a host containing a mutation in *glnA*. At 3 to 4 h after plating, the soft agar lawn of infected cells was UV irradiated with 220 μW/cm² for 15 s to stimulate recombination between phage and host. After overnight incubation, a single plaque was picked, suspended in 0.02 M Tris-hydrochloride (pH 7.5)–0.001 M Mg_2SO_4, chloroformed, diluted, and plated on ET3617 on Luria broth agar without added glutamine. Gln^+ phage formed turbid plaques, and recombinant Gln^- phage formed clear plaques. To obtain *glnL* and *glnG* mutations on the phage, λ *gln*64 was plated on strains containing *glnL* and *glnG* mutations and UV irradiated as described above. Single plaques were picked and plated on ET3617. The parental phage λ *gln*64 formed a clear plaque, whereas the recombinant phage, which were now Gln^+, formed turbid plaques. Since *glnL* and *glnG* mutations map under the deletion of λ *gln*64 or are very closely linked to the deletion, Gln^+ recombinant phages acquired the *glnL* or *glnG* allele with high frequency. Gln^+ recombinant phages were isolated at a frequency of 10^{-4} to 10^{-3}. All mutant phage were purified by two single-plaque isolations, and lysates were prepared by growing the mutant phage on the strain containing the same *gln* allele to ensure a homogeneous lysate. All mutant phage were tested to show they carried only a single mutation by crossing phages with strains containing various *gln* mutations. All of the constructed phage generated Gln^+ or Reg^+ recombinants with 25 mutants tested except those with the same allele.

Complementation tests. $RecA^-$ derivatives of Gln^- and Reg^- strains were constructed by transducing strains to Tet^r with P1 lysates grown on JC10240 (*srlC300*::Tn*10* *recA56*) and scoring transductants for UV sensitivity (8). Complementation tests were performed by spotting 5 μl of a λ *gln* mutant lysate (5×10^7 PFU/ml) together with an equal amount of λ *int*-h3 as helper on LBg plates spread with 0.1 ml of an overnight culture of various Rec^- Gln^- and Rec^- Reg^- strains. After overnight incubation at 34°C, plates were replicated to GN or GA plates to score the

Gln or Reg phenotype of the dilysogen. Plates were incubated for 2 days at 34°C to score the Gln phenotype and 5 days to score the Reg phenotype. Phenotypes were verified by further purification of transductants on selective plates and by determining presence of the λ *gln* transducing phage and the λ *int*-h3 phage by immunity to superinfection, since the two phages have different immunities. ET8000 could not be stably lysogenized by wild type λ, suggesting that it lacked *attB*. This was presumably the result of transduction of the strain to *hutC* from *Klebsiella aerogenes*. Therefore, λ *int*-h3 was used to generate dilysogens, since the λ *int*-h3 phage has an altered integrase which promotes lysogen formation in *attB*-deleted hosts 200 times as well as wild-type λ by increased lysogenization at secondary λ attachment sites (18).

RESULTS

Isolation of strains with point and insertion mutations in the *glnA* region. To genetically characterize the *glnA* region in *E. coli*, we isolated a large collection of independent Gln$^-$ (glutamine auxotroph) and Reg$^-$ (unable to utilize arginine or proline as nitrogen sources) mutants. Cultures of ET8000 were mutagenized with DES and phage Mu d1 to generate predominantly point and insertion mutations, respectively. Spontaneous and chemically induced mutations are presumed to be point mutations, although it is recognized that a minority may be small deletions or insertions. The Mu d1 phage carries the *lac* structural genes, Amp^r, and ends of Mu, enabling it to insert randomly in the host chromosome (6, 7, 29). These mutants are Lac$^+$ if the Mu d1 prophage is oriented so that transcription from the bacterial promoter proceeds into the *lac* end of Mu d1. Gln$^-$ and Reg$^-$ mutants were isolated by penicillin enrichment for inability to grow on arginine and proline as nitrogen sources. Additional Reg$^-$ mutants with spontaneous or Mu d1-induced suppressors of the Gln$^-$ phenotype of a *glnF* strain were isolated.

Mutations in all Gln$^-$ and Reg$^-$ strains were tested for linkage by P1 to *zig-2*::Tn*10*, an insertion that is 80% cotransducible with *glnA* on the *rha*-proximal side. Most Gln$^-$ mutants had mutations linked to this insertion. A total of 87 Gln$^-$ mutants were isolated; 27 contained DES-induced mutations, and 60 contained Mu d1- or Mu-induced mutations. About 8% of the Gln$^-$ and 6% of Reg$^-$ mutants isolated from Mu d1-infected cultures had a Mu-induced *gln* mutation, since the Mu d1 lysate is a mixture of Mu d1 and Mu as helper. Of the Reg$^-$ mutants isolated as unable to use arginine and proline, 60% had mutations linked to *glnA*. Most of the Gln$^-$ and Reg$^-$ strains with mutations unlinked to *glnA* had mutations that were probably near *glnF*, *glnD*, or *gltB* because they were recessive to F-prime factors carrying these regions of the *E. coli* chromosome. The map position of mutations not linked to *glnA* nor recessive to these episomes was not pursued. Only Gln$^-$ and Reg$^-$ mutants with *glnA*-linked mutations were analyzed in this study. A total of 290 Reg$^-$ mutants with *glnA*-linked mutations were isolated. Among mutants isolated as unable to utilize arginine and proline, 120 contained DES-induced, 85 contained Mu d1- or Mu-induced, and 14 contained spontaneous mutations. A total of 71 mutants were selected as *glnF* suppressors; 23 contained Mu d1-induced mutations, and 48 contained spontaneous mutations.

Isolation of deletion mutants of λ *gln* and use in mapping *gln* mutations. Deletion mutants of λ *gln* were isolated as described in Materials and Methods by selection for heat- or chelating agent-resistant mutants, since inactivation of λ by heat or low ionic strength depends on the DNA content of the λ head (22). We used this approach to select deletion mutants of λ *gln* (NM811), a λ-specialized transducing phage containing a *Hin*dIII fragment which includes the *glnA* region (14). The 73 independent deletion-containing derivatives of λ *gln* were crossed with Gln$^-$ and Reg$^-$ strains. The wild-type λ *gln* and λ *trpE* phages were used as positive and negative controls. A mutation was defined to be outside a deletion if it gave more transductants with the deletion-containing phage than that observed with the λ *trpE* phage, which detects the reversion rate of each mutation. Typically, no revertants were seen. A positive result by this method yielded from 50 colonies to a confluent patch of transductants on the selective medium, which determined an unambiguous map position of the mutations.

Figure 1 shows the genetic map of *glnA*. A total of 27 DES-induced and 60 Mu d1- or Mu-induced mutations in strains isolated in this study were mapped. We also mapped one Tn*5* insertion and five hydroxylamine-induced, two EMS-induced, and nine spontaneous *glnA* mutations in strains previously isolated. A total of 104 mutations, 43 points and 61 insertions, were divided into 24 deletion groups by 39 deletions. Deletions obtained from Mu d1-induced *glnA-lac* fusions were used to show that transcription is counter-clockwise on the *E. coli* map and left to right in Fig. 1 (16). Two-dimensional polyacrylamide gel analysis of extracts from 40 strains containing *glnA* point mutations showed that 60% made the inactive GS polypeptide and most showed charge alterations (G. Roberts, personal communication). Strains which produced charge-altered GS had mutations that mapped from deletion interval 4 to 19, which indicated that this entire region was required to code for GS.

Three strains with the Mu d1-induced muta-

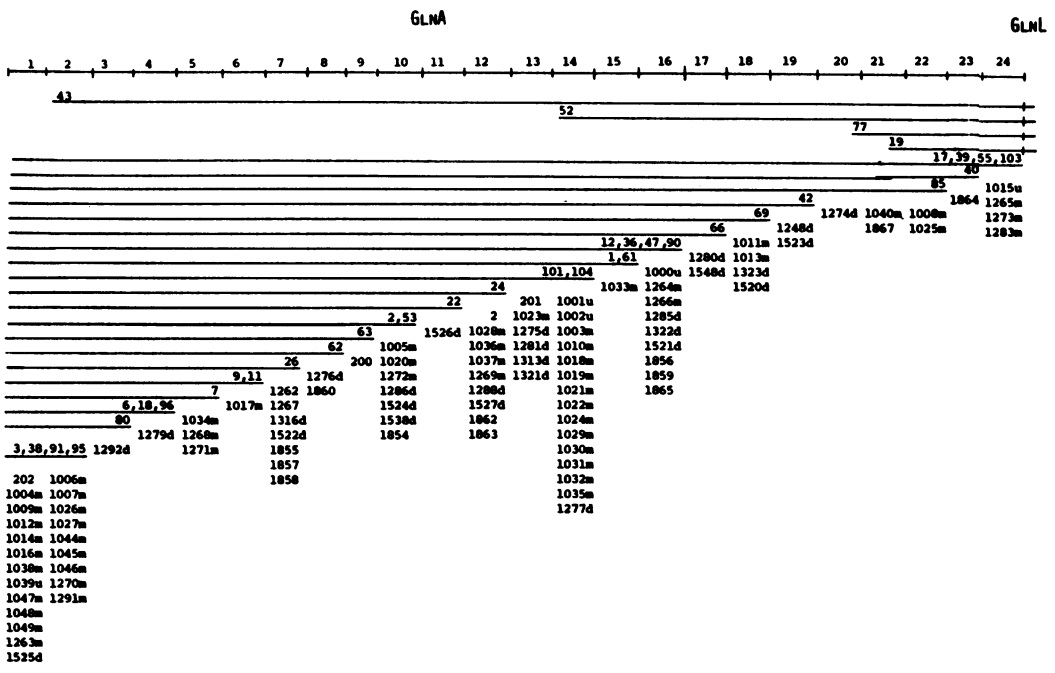

FIG. 1. Map of *glnA* mutations. Deleted DNA in λ *gln* derivatives is indicated by solid lines. Phages are numbered at their deletion endpoint in *glnA*. Deletion intervals are numbered 1 through 24. Vertical columns list mutations which map in that interval. Mutations are DES(d)-, Mu d1(m)-, or Mu(u)-induced or spontaneous (no letter) unless defined differently in the text. The *rha* locus on the *E. coli* chromosome is to the left of *glnA* as the figure is read. Deletions in λ *gln*43, λ *gln*52, λ *gln*77, and λ *gln*19 extend through *glnL* and *glnG*.

tions *gln-1044*, *-1263*, and *-1270* located in deletion intervals 1 and 2 of *glnA* reverted to Gln^+ at a frequency of 10^{-8} to 10^{-7}. Four mutants with the insertions *gln-1047*, *-1048*, *-1049*, and *-1291* which mapped in the same deletion intervals had a leaky Gln^- phenotype. All other Mu d1-induced mutations in *glnA* had a tight Gln^- phenotype, and reversion was not observed when 10^9 cells were plated on minimal medium without glutamine. Mutations induced by Mu or Mu d1 result in the inactivation of the gene and loss of its product (29). Mu-induced mutations revert by precise excision at a frequency of less than 10^{-10} (29). A higher reversion frequency is obtained when the original mutation is suppressed by a second site mutation (5). Therefore, the leaky and reverting phenotypes of these seven strains are unexpected. Their phenotypes and map positions suggest that these mutations may be located just outside the structural gene in the control region for *glnA*. Insertions in a position which do not eliminate all *glnA* expression result in leaky Gln^- mutants. Reversion to Gln^+ may arise by creation of a new promoter.

Figure 2 shows the map of mutations in Reg^- strains. In addition to mapping mutations in strains isolated during this study, we also mapped two hydroxylamine-induced, two Mu-induced, and one Tn*5*-induced mutation in previously isolated Reg^- strains. A total of 295 mutations were divided into 22 deletion intervals by 38 deletions. A total of 131 mutations were in *glnL*, and 164 were in *glnG*, the two genes defined by complementation analysis. *glnL* contained 86 Mu d1-, 5 Mu-, and 29 DES-induced mutations and 11 spontaneous mutations. *glnG* contained 17 Mu d1-, 2 Mu-, 91 DES-, 1 Tn*5*-, and 2 hydroxylamine-induced mutations and 51 spontaneous mutations. Mutations generated by all of the selection methods were represented in both genes, although *glnL* contained the majority of Mu d1 insertions and *glnG* the majority of point mutations. We found that 35 of 62 Gln^- mutants which were isolated by enrichment for the Gln^- phenotype after chemical mutagenesis contained mutations which mapped in *glnG*. These 35 point mutations mapped between λ *gln*88 and λ *gln*79. Although mutations conferring a Reg^- Gln^- phenotype mapped in *glnG*, no mutations which conferred only a Reg^- phenotype mapped in *glnA*.

Complementation analysis. Complementation tests were performed between mutations in *glnA*, *glnL*, and *glnG* to investigate the transcriptional organization of these three genes.

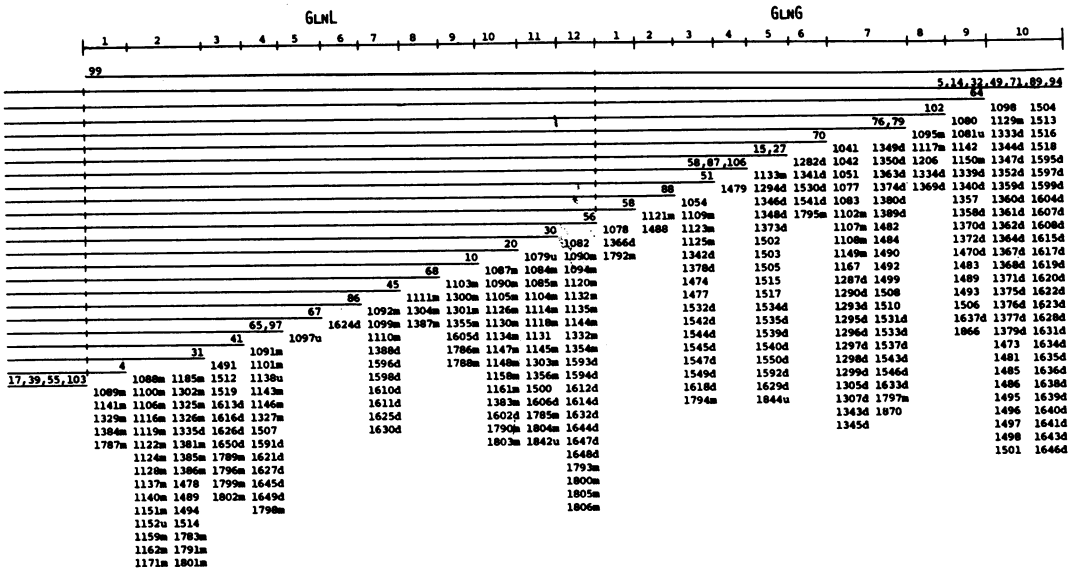

FIG. 2. Map of *glnL* and *glnG* mutations. All notations are as described in the legend to Fig. 1. The division between *glnL* and *glnG* was determined by complementation analysis. Deletion intervals are numbered at the top of the figure. Deletions in all phage except λ *gln*99 extend through *glnA*.

First, 7 *glnA*, 11 *glnL*, and 8 *glnG* mutations were recombined into the λ *gln* transducing phage as described in Materials and Methods. Complementation tests were performed by construction of strains which were diploid for the *gln* region. Complementation analysis was performed on mutations which mapped throughout *glnA*, *glnL*, and *glnG* and included 32 of 104 *glnA*, 40 of 131 *glnL*, and 45 of 164 *glnG* mutations. Rec$^-$ strains with various *gln* mutations were infected with λ *gln* phages which also contained a *gln* mutation in the presence of a helper phage, λ *int*-h3, to ensure phage integration into a λ attachment site. The dilysogens were tested for growth in the absence of glutamine (Gln phenotype) and for growth on arginine (Reg phenotype) by streaking strains on GN and GA plates. The presence of both phage was verified by immunity to λ *gln* (*imm-21*) and λ *int*-h3 (*imm* λ).

GlnA. Table 2 shows the complementation results between several *glnA* mutations for the Gln and Reg phenotypes. No Gln$^+$ complementation was observed in merodiploids in which one or both *glnA* alleles came from a parent which failed to synthesize detectable GS polypeptide on two-dimensional polyacrylamide gels. This was the expected result for mutations located in a single gene. All *glnA* mutations tested from strains which produced an inactive GS detectable on gels showed intragenic complementation with two or more different *glnA* mutations. Intragenic complementation occurred most frequently between mutations which mapped far apart (for example, *A1279/A1274* and *A1525/A1523*) and less frequently between mutations which mapped near each other (for example, *A1279/A1538*) on the *glnA* deletion map shown in Fig. 1. The merodiploids formed between *glnA1280* and *glnA1538* and between *glnA1279* and *glnA1525* gave weak complementation for the Gln phenotype and negligible complementation for the Reg phenotype. All other examples of intragenic complementation were expressed equally well for the Gln and Reg phenotypes. Since GS functions as a dodecamer, it is not surprising to find mutations in *glnA* which yield a merodiploid capable of producing active GS protein.

Mutations in Reg$^-$ strains formed two complementation groups (Table 3). Most λ *gln* phages with point mutations which mapped between λ *gln*17 and λ *gln*56 failed to complement other mutations which mapped in this region. This complementation group was designated *glnL*. Most point mutations in *glnL* complemented mutations which mapped to the right of the deletion of λ *gln*56. Mutations in this region failed to complement each other, and this complementation group was designated *glnG*.

GlnL. *glnL* point mutations formed two classes defined by complementation analysis. Class I *glnL* point mutations complemented most *glnG* mutations (Table 3). Mu d1-induced mutations and Class II point mutations in *glnL* failed to complement *glnG* mutations. Since Mu-induced mutations are known to be polar (13), the failure of Mu d1-induced mutations to complement

TABLE 2. Complementation analysis among *glnA* mutations[a]

Recipient	Donor							
	1279	*1280*	*1281*	*1521*	*1522*	*1524*	*1525*	*gln*$^+$
glnA points, Class I								
1274, 1280, 1281, 1285, 1288, 1322, 1323, 1523	+	−	−	−	−	−	+	+
1279	−	+	+	−	−	−	W	+
1525	W	+	+	−	−	−	−	+
1538	+	W	−	−	−	−	+	+
glnA points, Class II								
1275, 1292, 1316, 1521, 1522, 1524	−	−	−	−	−	−	−	+
glnA Mu d1 insertions								
1006, 1014, 1017, 1019, 1020, 1028, 1040, 1044, 1262,[b] *1264, 1265, 1267, 1271, 1272, 1286*	−	−	−	−	−	−	−	+

[a] Complementation tests were performed as described in the text by infecting Rec$^-$ recipients containing the *glnA* alleles listed with λ *gln* derivatives containing the indicated donor alleles. Growth of lysogens on GN (Gln phenotype) was scored. Complementation for Gln and Reg phenotypes were identical unless described differently in the text. +, Approximately wild-type growth; W, weak growth; −, no growth of lysogens on selective medium.

[b] Tn*5* insertion.

glnG mutations indicates that these two genes form an operon which is transcribed away from *glnA* as Fig. 2 is read. Like *glnL*::Mu d1 mutations, Class II *glnL* mutations failed to complement *glnG* mutations, suggesting that they too are polar and may represent deletions, insertions, or nonsense or frameshift mutations. Class I *glnL* point mutations are considered to be nonpolar since they show no reduction in the ability to complement *glnG* mutations. Although not shown in Table 3, there were some instances of intragenic complementation between *glnL* Class I mutations which mapped in the promoter-distal portion of *glnL*. *glnL1388* and *glnL1598* complemented *glnL1593* and *glnL1632* and *glnL1598* complemented the *glnL* alleles *1593, 1594, 1602, 1606, 1612, 1614, 1632, 1644, 1647,* and *1648*. The presence of intragenic complementation suggests that this gene product may function as a multimeric complex.

GlnG. *glnG* point mutations formed three classes based on complementation tests. All Mu d1-induced mutations and most point mutations in *glnG* (Class I) complemented nonpolar point mutations in *glnL*. Class II *glnG* point mutations mapped in the *glnL*-proximal two-thirds of *glnG*, complemented *glnL* mutations poorly, and had varying degrees of glutamine auxotrophy. Class III *glnG* mutations complemented very poorly and mapped predominantly in the *glnL*-distal half of *glnG*. Unlike Class II mutations, they were not at all *Gln*$^-$. Class II *glnG* mutations were complemented well by λ *gln* wild type, but Class III *glnG* mutations were not, suggesting that they have a partially transdominant negative phenotype.

Complementation between *glnA* and *glnL* or *glnG* mutations. When strains carrying insertions or point mutations in *glnA* were infected with λ *gln* phages containing *glnL* or *glnG* mutations, all resulting merodiploids grew well in the absence of glutamine, indicating complementation for the Gln phenotype. Table 3 shows the complementation results obtained when these dilysogens were tested for growth on glucose-arginine medium. *glnA* mutations from strains which synthesized a detectable, inactive GS protein complemented *glnL* and *glnG* mutations well, except Class II and Class III *glnG* mutations. Since Class II mutations are Gln$^-$ and Class III mutations are somewhat transdominant, they might be expected to have altered regulatory properties affecting *glnA* expression and therefore not to complement *glnA* mutations well. Mu d1-induced mutations in *glnA* and mutations in strains which do not synthesize a detectable GS polypeptide failed to complement *glnG* and most *glnL* mutations. These mutations did complement four nonpolar *glnL* Class I mutations, *1514, 1650, 1591,* and *1605*, but not *1598, 1593,* or *1632*. When λ *glnL*$^-$ phages were used to infect those *glnA* strains, the growth on GA medium was poorer than that obtained when the recipient was a GlnA$^-$ strain which produced detectable polypeptide. In part, these results suggest that *glnA* is polar onto *glnL* and *glnG*, since *glnA* insertions failed to complement *glnG* mutations and many *glnL* mutations for the Reg

TABLE 3. Complementation analysis among *glnA*, *glnL*, and *glnG* mutations for growth on arginine as nitrogen source[a]

Recipient	Donor							
	glnA, Class I *1279, 1280, 1281, 1525*	*glnA*, Class II *1521, 1522*	*glnL*, Class I *1514, 1591, 1593, 1598, 1605, 1632, 1650*	*glnL*, Class II *1082, 1478, 1491, 1500*	*glnG*, Class I *1342, 1361, 1486, 1488, 1501*	*glnG*, Class II *1533*	*glnG*, Class III *1629, 1637*	*gln*$^+$
glnA[b]								
Points, Class I	See Table 2	−	+	+	+	VW	VW	+
Points, Class II	−	−	W	−	−	−	−	+
Mu d1 insertions	−	−	W	−	−	−	−	+
glnL								
Points, Class I[c]	+	−	−	−	+	W	W	+
Points, Class II[d]	+	−	−	−	−	−	−	+
Mu d1 insertions[e]	+	−	−	−	−	−	−	+
glnG								
Points, Class I[f]	+	−	+	−	−	−	−	+
Points, Class II[g]	W	−	W	−	−	−	−	+
Points, Class III[h]	VW	−	VW	−	−	−	−	W
Mu d1 insertions[i]	+	−	+	−	−	−	−	+

[a] Complementation tests were performed as described in the text by infecting Rec$^-$ recipients containing the *gln* alleles listed with λ *gln* derivatives containing the indicated donor alleles. +, Approximately wild-type growth; W, weak growth; VW, very weak growth; −, no growth of lysogens on GA medium.

[b] All *glnA* alleles are listed in Table 2.

[c] *glnL* point mutations, Class I, are *1388, 1514, 1591, 1593, 1594, 1598, 1602, 1605, 1606, 1612, 1613, 1614, 1632, 1644, 1647, 1648*, and *1650*.

[d] *glnL* point mutations, Class II, are *1082, 1478, 1491*, and *1500*.

[e] *glnL* Mu d1-induced mutations are *1088, 1089, 1090, 1091, 1094, 1105, 1120, 1132, 1135, 1141, 1144, 1332, 1381, 1384, 1354, 1793, 1800, 1805*, and *1806*.

[f] *glnG* point mutations, Class I, are *1078, 1335, 1341, 1342, 1359, 1361, 1366, 1374, 1473, 1479, 1485, 1486, 1488, 1501, 1504, 1513, 1518, 1604, 1628, 1636*, and *1643*.

[g] *glnG* point mutations, Class II, are *1287, 1290, 1294, 1530, 1533, 1535, 1537, 1539, 1541*, and *1546*.

[h] *glnG* point mutations, Class III, are *1352, 1371, 1495, 1497, 1599, 1619, 1622, 1629*, and *1637*.

[i] *glnG* Mu d1-induced mutations are *1107, 1121, 1129, 1133*, and *1792*.

phenotype. The ability to obtain significant complementation between *glnA* insertion mutations and some *glnL* mutations suggests that at least some *glnL* expression occurs independently of the *glnA* promoter.

When we attempted to construct the merodiploids in the reciprocal orientation by infecting *glnL* Class I recipients with λ *gln* phages with polar point mutations from GlnA$^-$ strains, we were unable to detect any growth when phage-infected cells were replicated to GA medium (Table 3). For the experiments described in the paragraph above, the recipient carried a *glnA* mutation, and we were able to isolate the merodiploid by selecting Gln$^+$ dilysogens. Purified merodiploids were then tested for GA growth. These somewhat different methods may explain the nonidentical results for these two reciprocal crosses in Table 3. In all other reciprocal crosses in Table 3, results were identical.

GlnC mutants. Strains defective in *glnD* lack uridylyltransferase and uridylyl-removing enzyme (4, 9). In *E. coli*, GlnD$^-$ strains have highly adenylylated GS under all conditions, fail to derepress GS under nitrogen-poor conditions, and grow poorly in the absence of glutamine (9). Revertants with *glnA*-linked mutations which suppress the GlnD$^-$ phenotype have been isolated, and they produce high levels of GS in the presence of ammonia, the GlnC phenotype (4, 9).

Strains with mutations in *gltB*, the gene for glutamate synthase, are unable to use a wide variety of nitrogenous compounds such as arginine, ornithine, and γ-aminobutyrate (21). Among revertants selected for ability to use these nitrogen sources were those with the GlnC phenotype, in which the mutation responsible for the GlnC phenotype was linked to *glnA* (21).

To obtain a collection of GlnC strains, we isolated spontaneous revertants of ET8053 (*glnD99*::Tn*10*) and ET8050 (*gltB31*) by selection of mutants which could use arginine as a nitrogen source. A colony test developed from the γ-glutamyltransferase assay was used to identify GlnC mutants, those revertants which synthesized derepressed levels of GS in the presence of ammonia. Since the GlnC strains do not have a negative phenotype, deletion mapping by positive selection could not be performed. Therefore, we developed an alternative approach to map the mutations from the GlnC strains. We isolated a set of chromosomal deletions which have a well-defined endpoint in *glnA*, *glnL*, or *glnG*. These were constructed by introducing a *rha*::Mu mutation into strains containing Mu d1 insertions in *glnA*, *glnL*, or *glnG* which were mapped by the λ *gln* deletion phages. Homologous recombination between Mu and Mu d1 results in a deletion with one endpoint in *rha* and the other endpoint at the site of the Mu d1 insertion in the *gln* genes (16). The *metB*::Tn*5* and the *glnD99*::Tn*10* mutations were transduced into four Δ(*rha-gln*) strains. Strains ET10300, ET10575, ET10574, and ET10573 contain deletions in which one endpoint is determined by *glnA1272* (deletion interval 10), *glnL1329* (deletion interval 1), *glnL1135* (deletion interval 12), and *glnG1108* (deletion interval 7), respectively. When these deletions are transduced to Rha$^+$ with P1 grown on GlnC strains, all Rha$^+$ transductants will be GlnC if the suppressor mutation maps under the deletion. If the mutation maps outside the deletion, one would expect to find some recombinants which lack the GlnC allele and phenotype. Since the recipient contained the *glnD99*::Tn*10* mutation from which the revertants were selected, Rha$^+$ transductants can simply be scored for growth on arginine to determine whether the *glnD* suppressor mutation is present in the transductant. The recipients also have the mutation *metB*::Tn*5*. The order of the markers in this region of the chromosome is *met-rha-gln*. Since *glnA* is about 2 min from *metB*, selection of Met$^+$ Rha$^+$ transductants increased the relative frequency of the second crossover in the *gln* region.

P1 was grown on 15 GA$^+$ revertants of ET8053 containing mutations conferring the GlnC phenotype listed in Table 4 and used to transduce the four deletion strains to Met$^+$ Rha$^+$ on rhamnose-ammonia-glutamine minimal medium. All transductants were tested for the ability to grow on arginine as a nitrogen source. Table 4 shows the number of arginine utilizers (GA$^+$) and nonutilizers (GA$^-$) from these crosses. The numbers of transductants obtained were low because the combined distance from *metB* to *rha* to *glnG* is large. All GA$^+$ transductants produced high levels of GS in the presence of ammonia. All GA$^-$ transductants had a leaky Gln$^-$ phenotype typical of the parental *glnD* strain. Table 4 shows that most Rha$^+$ Met$^+$ transductants of deletion-containing recipients are GA$^+$, indicating linkage of the GlnC mutation to *glnA*. In a control transduction with a P1 lysate grown on wild-type ET8000, no GA$^+$ transductants were obtained.

All GlnC mutations gave GA$^-$ recombinants with a deletion that ends in the middle of *glnA* (ET10300), indicating that all of the mutations must map to the right of *glnA1272* on the *glnA* map of Fig. 1. All except three alleles (*gln-1210*, *-1213*, and *-1216*) gave GA$^-$ recombinants with a deletion generated from a Mu d1 insertion in the first deletion interval of *glnL* (ET10575). These results indicate that 12 of 15 mutations responsible for suppression of *glnD99*::Tn*10* and the GlnC phenotype are not in *glnA* but in the

TABLE 4. Mapping mutations in GlnC strains with chromosomal *rha-gln* deletions

gln allele in GlnC strains[a]	Recipient							
	ET10300 Δ(*rha-glnA*) 1693_{10}[b]		ET10575 Δ(*rha-glnL*) 1821_{1}[b]		ET10574 Δ(*rha-glnL*) 1711_{12}[b]		ET10573 Δ(*rha-glnG*) 1705_{7}[b]	
	GA⁻	GA⁺	GA⁻	GA⁺	GA⁻	GA⁺	GA⁻	GA⁺
1207	20	36	6	73	0	58	0	10
1208	10	250	1	100	0	60	0	56
1209	7	21	12	118	4	122	0	37
1210	5	122	0	120	0	90	0	65
1213	8	210	0	85	0	130	0	63
1214	13	90	4	105	0	27	0	24
1216	1	116	0	130	0	95	0	60
1218	1	13	13	112	4	63	0	12
1219	2	38	3	148	0	48	0	24
1222	16	140	2	67	0	108	0	63
1223	8	16	13	81	0	64	0	16
1224	4	55	5	135	0	155	0	20
1228	5	16	3	126	3	114	0	10
1231	7	34	11	97	0	109	0	18
1232	2	20	4	121	2	114	0	15
gln⁺	25	0	25	0	25	0	10	0

[a] P1 grown on GlnC strains containing the listed alleles was used to transduce four recipient strains which are *metB136*::Tn*5* *glnD99*::Tn*10* Δ(*rha-gln*) to Met⁺ Rha⁺. All transductants were tested for growth on GA medium. GA⁻ transductants did not grow on arginine as a nitrogen source, had not acquired the *glnD* suppressor mutation, and were not constitutive for GS expression. GA⁺ transductants grew on arginine as a nitrogen source, had acquired the *glnD* suppressor, and had the GS constitutive phenotype.

[b] Subscripted number indicates the deletion interval location of the deletion endpoint in *glnA*, *glnL*, or *glnG*.

adjacent region *glnL* or *glnG*. The remaining three alleles may map in the promoter-distal end of *glnA* or very early in *glnL*. Four alleles gave GA⁻ recombinants with ET10574, which has a deletion endpoint at the *glnL-glnG* border, suggesting that these mutations map in *glnG* or at the very end of *glnL*.

A limited number of similar crosses were performed with GlnC strains obtained as suppressors of *gltB31*. The results are similar to those obtained from *glnD* suppressors, indicating that 8 of 11 mutations tested mapped outside of *glnA* in the regulatory genes *glnL* and *glnG*. GlnC mutants obtained as suppressors of *glnD99*::Tn*10* and *gltB31* could not suppress the glutamine auxotrophy conferred by *glnF* mutations, indicating that expression of the GS constitutive phenotype requires an intact *glnF* gene.

DISCUSSION

We isolated Gln⁻ and Reg⁻ strains of *E. coli* which contained DES, Mu d1, and spontaneous mutations in the *glnA* region. To map these and other mutations, a collection of 73 overlapping deletions was obtained on λ *gln*-specialized transducing phages. A fine structure map of 104 *glnA*, 131 *glnL*, and 164 *glnG* mutations was determined.

Complementation analysis was performed between mutations from Gln⁻ and Reg⁻ strains. Mutations conferring the Reg⁻ phenotype were described as a single gene, called *glnG* in *E. coli* (20) and *glnR* in *S. typhimurium* (15). We found that these mutations formed two complementation groups, designated *glnL* and *glnG*. These two genes probably correspond to *ntrB* and *ntrC*, recently defined by McFarland et al. (17). We mapped *gln-1842*::Mu and *gln-1844*::Mu (formerly *gln-2* and *gln-4*, respectively), two of three mutations conferring the Reg⁻ phenotype which were originally used to define *glnG* (20). *gln-1844* mapped in *glnG*, and *gln-1842* mapped in *glnL* (Fig. 2).

Complementation tests between insertions and point mutations were used to define the transcriptional organization of these three genes. Insertion mutations and Class II point mutations (Table 3) in *glnL* failed to complement *glnG* mutations, indicating that these *glnL* mutations were polar onto *glnG* and that these two genes formed an operon in which transcription proceeds from *glnL* into *glnG*. McFarland et al. did not find polarity of *ntrB* mutations onto *ntrC*, even though mutations were induced by ICR-191 and might be expected to be polar, frameshift mutations (17).

On the basis of several observations, we believe that transcription of the *glnL-glnG* operon can originate in two ways: (i) by read-through into *glnL* and *glnG* of transcription initiated at the *glnA* promoter and (ii) by transcription initiated at the *glnL-glnG* promoter. We found that

insertions in *glnA* complemented *glnL* and *glnG* mutations for the Gln phenotype but not for the Reg phenotype (growth on arginine as a nitrogen source). Pahel and Tyler found that insertions which we have shown to be in *glnL* and *glnG* complement insertions in *glnA* for the Gln phenotype and for GS regulation in response to nitrogen availability but do not complement for the Reg phenotype (20). The polarity for the Reg phenotype suggests that transcription may proceed from *glnA* into *glnL* and *glnG* under certain conditions. However, the ability of *glnA* insertions to complement *glnL* and *glnG* mutations for GS regulation indicates that sufficient *glnL* and *glnG* products are being produced in the absence of transcription from *glnA*. We found that β-galactosidase levels in *glnL-lac* and *glnG-lac* fusions were not reduced when *glnA*::Tn*5* was introduced into the *lac* fusion strains, indicating that transcription must be initiated between *glnA* and *glnL* (D. MacNeil, unpublished observations). Also, the observation that *glnA* insertion mutations complement some *glnL* Class I point mutations for growth on arginine suggests that some transcription of the *glnL-glnG* operon must originate independently of *glnA* expression. Therefore, the failure of *glnA* insertion mutations to complement *glnL* and *glnG* mutations for the Reg phenotype may reflect termination of transcription which ordinarily reads through into *glnL* and *glnG*. This failure may also reflect altered regulation of a promoter between *glnA* and *glnL* in the presence of mutant *glnL* and *glnG* products which differ from the wild-type products in function or quantity or both. The relative contribution of transcription from the *glnA* and *glnL* promoters in wild-type cells under various conditions cannot be determined here.

glnA, *glnL*, and *glnG* are transcribed in the same direction, away from the *rha* locus (left to right as Fig. 1 and 2 are read). The direction of transcription of these three genes determined by complementation analysis was the same as that determined by analysis of phenotypes of deletion-containing strains obtained from Mu-Mu d1 dilysogens in which Mu d1 insertions in *glnA*, *glnL*, and *glnG* were used (16). These results are also in agreement with other determinations of the direction of *glnA* transcription in *E. coli* (25) and *K. aerogenes* (31). Recent results of Backman et al. (1) indicate that the direction of transcription of *glnA* and *glnG* is the same as that determined in this work.

Many examples of intragenic complementation for Gln and Reg phenotypes were observed between *glnA* mutations from strains capable of producing an inactive GS polypeptide visible on two-dimensional gels. Intragenic complementation is expected among *glnA* mutations, since GS functions as a multimeric complex. GS proteins produced by complementing mutations may be useful for investigation of altered structural and functional properties of an enzyme produced by two mutant *glnA* alleles. Mutations in Gln$^-$ strains which failed to produce detectable GS polypeptide failed to complement *glnL* and *glnG* mutations and did not show intragenic complementation. These are most likely polar mutations.

The two complementation classes of point mutations in *glnL* represent nonpolar and polar mutations. Intragenic complementation was also observed between some Class I *glnL* mutations which map at the promoter-distal end of *glnL*. In *glnG*, three classes of point mutations, based on complementation tests, could be distinguished. Mu d1-induced mutations and most point mutations (Class I) complemented nonpolar *glnA* and *glnL* mutations well. Class II mutations complemented *glnA* and *glnL* mutations poorly and conferred varying degrees of glutamine auxotrophy. Another group of *glnG* mutations, Class III, exhibited a transdominant negative phenotype in that they were not well complemented by wild-type, *glnA*, or *glnL* mutations. The results suggest that Class II and III *glnG* mutations cause production of mutant proteins that are altered in their regulatory properties. Since both classes showed poor complementation with *glnA* and *glnL* mutations, it is possible that the *glnG* product is involved in regulating expression of *glnA* and *glnL*.

GlnD$^-$ strains produce little GS and fail to grow on poor nitrogen sources (4). Revertants with *glnA*-linked suppressors of the *glnD* phenotype were isolated in *K. aerogenes* (9). They produce GS constitutively in the presence of ammonia and are thought to be in *glnA* (9). We isolated 15 GlnC mutants as suppressors of a *glnD* strain. Strains containing large deletions with one endpoint in *rha* and the other at a known location in *glnA*, *glnL*, or *glnG* were used to map GlnC mutations. With these deletions, the ability to obtain recombinants which are not GlnC as the result of a crossover between a deletion and the suppressor mutation indicates that the suppressor cannot map under the deletion. However, the inability to obtain such recombinants suggests that the mutation maps under the deletion; however, it may indicate that, owing to the close linkage of the GlnC mutation to the deletion, an insufficient number of transductants were obtained to detect the non-GlnC recombinant. Therefore, the experiments reported here allow us to say that most of the GlnC mutations tested clearly map outside of *glnA*; these are probably in *glnL*, but some may be in *glnG*. Backman et al. (1) found that a plasmid containing *glnA* and *glnG* with a 480-

base-pair deletion between *glnA* and *glnG* (in the region we have genetically defined as *glnL*) led to a GlnC phenotype in a strain deleted for the *glnA-glnG* region. However, the constitutive phenotype of this strain may not be due solely to the deletion mutation but may result from gene dosage effects due to the multicopy nature of the plasmid.

Based on the genetic analysis of mutations in Gln^-, Reg^-, and GlnC strains presented here, it is clear that the multitude of phenotypes ascribed to mutations in *glnA* (2, 3, 9, 10, 27) were probably due to mutations in three genes, *glnA*, *glnL*, and *glnG*. The two genes adjacent to *glnA* are regulatory genes, since mutations in these genes affect the expression of *glnA* and other nitrogen-regulated genes. Previous mapping by three-factor reciprocal crosses showed that mutations in Reg^- (GlnR) and GlnC strains mapped among mutations from Gln^- strains in *K. aerogenes* (10, 27). Our results show that mutations leading to a Gln^- phenotype can result from mutations in *glnA* and also in *glnG*. Mutations leading to a GlnC phenotype result from mutations not in *glnA*, but probably in *glnL*. Mutations in both *glnL* and *glnG* can lead to the Reg^- phenotype. Thus, *glnL* and *glnG* may account for all aspects of regulation formerly attributed to the GS protein itself. We are now using the fine structure map of these genes to correlate the phenotypes of mutations with their map positions to study the functions of these genes.

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